

Heat Tolerance in the Black Abalone, *Haliotis cracherodii* Leach, 1814: Effects of Temperature Fluctuation and Acclimation

BY

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(3 Text figures)

INTRODUCTION

THE CORRELATION of higher thermal tolerance with higher vertical distribution of intertidal mollusks is well known (e.g., DAVIES, 1970; FRAENKEL, 1968; SANDISON, 1968; WOLCOTT, 1973; and many others). However, examinations of temperature as a limiting environmental factor should consider the effects of the acclimation temperature and of temperature fluctuations during the tidal cycle on the thermal tolerance of an intertidal organism. The black abalone, *Haliotis cracherodii* Leach, 1814, is common in the intertidal zone of California at levels of 0.3 to 1.0 meters above mean lower low water, but its thermal tolerance has not been reported previously. Although the effects of temperature on the larval development rate of several other species of abalone from California have been studied (LEIGHTON, 1972; 1974), the thermal tolerance of adults of only red abalone, *Haliotis rufescens* Swainson, 1822, has been investigated (EBERT, 1974). The present study provides comparative information on the heat tolerance of adult black abalone, which were tested for 96 hours to determine the temperature at which 50 percent of the sample survived, i.e., the median effective temperature (ET₅₀).

The ability of the abalone to hold onto a substrate was used as the criterion for irreversible thermal damage in these experiments, because it is difficult to assess physiological death in this animal. Animals acclimated to both 11°C and 16°C were tested to provide information on

seasonal changes in thermal tolerance of *Haliotis cracherodii*. Abalone in the experiments were either continuously submerged in heated water or periodically exposed to cooler air. Measurements of mortality rates at constant test temperatures provided standardized comparisons of thermal tolerance of the abalone, but this provided rather unrealistic experimental conditions. In the field, the tidal cycle would impose fluctuating temperatures, with stressful periods lasting about 6-12 hours. Therefore, the response of abalone exposed to repeated, short intervals of thermal stress was compared to their response to long, constant stress. Although intertidal organisms in central California are usually exposed to fluctuations of warm air and cool seawater, the experimental design of using heated test water and cooler air helped eliminate the variable of desiccation during heat stress. Because the fluctuating thermal regimes produced much lower "heat doses" than the constant conditions, and because black abalone were not expected to have evolved physiological mechanisms for tolerating continuous stress for as long as 96 hours, we predicted that abalone in fluctuating regimes would have better survivorship than those in constant conditions. Although the present report confirms this prediction, we found that the response to heat stress in black abalone was extremely abrupt once lethal temperatures were reached, and that exposure to a fluctuating regime produced only a small increase in the ET₅₀ value.

MATERIALS AND METHODS

This study was conducted at the Pacific Gas and Electric Company Thermal Effects Laboratory at the Diablo Canyon Nuclear Power Plant on the central California coast of San Luis Obispo County. Large (10-15 cm shell length)

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black abalone collected in the Diablo Canyon area were acclimated to either 11.5 or 16°C in the laboratory for at least one week prior to the start of the experiment. During the experiment, abalone from each of the two acclimation regimes were maintained either continuously submerged or held in a cycle of six hours submergence alternating with six hours exposure to air in laboratory tanks, which were equipped with a machine that automatically raised and lowered their water level. Thus, there were 4 experimental groups: 11°C acclimated tidal and non-tidal abalone, and 16°C acclimated tidal and non-tidal abalone. There were 2 phases to the experiment. The first phase during June, 1978, measured the thermal tolerance of 16°C acclimated non-tidal abalone moving freely on the bottom of the tanks. The second phase during August, 1978, measured the thermal tolerance of 16°C tidal, 11°C tidal, and 11°C non-tidal abalone held in trays, which positioned the animals at a constant level in the simulated tidal cycle.

A supplemental experiment was run to determine the core body temperature of 13-14 cm long abalone during exposure to air following heat stress. Following immersion in seawater at 25°C for 2 hours, the seawater was drained and the abalone were exposed to air at 19-20°C for 6 hours. At successive intervals during this period, the core body temperatures of two abalone were measured by removing them from the substrate and quickly inserting a thermistor probe through the foot into the center of a body mass. The temperature of each abalone was measured only once, and the animals were discarded. During exposure to air, the abalone cooled rapidly, and within 2 hours their core body temperatures equaled the air temperature $\pm 0.2^\circ\text{C}$. Core body temperatures remained within $\pm 0.3^\circ\text{C}$ of the air temperature for the rest of the 6-hour period.

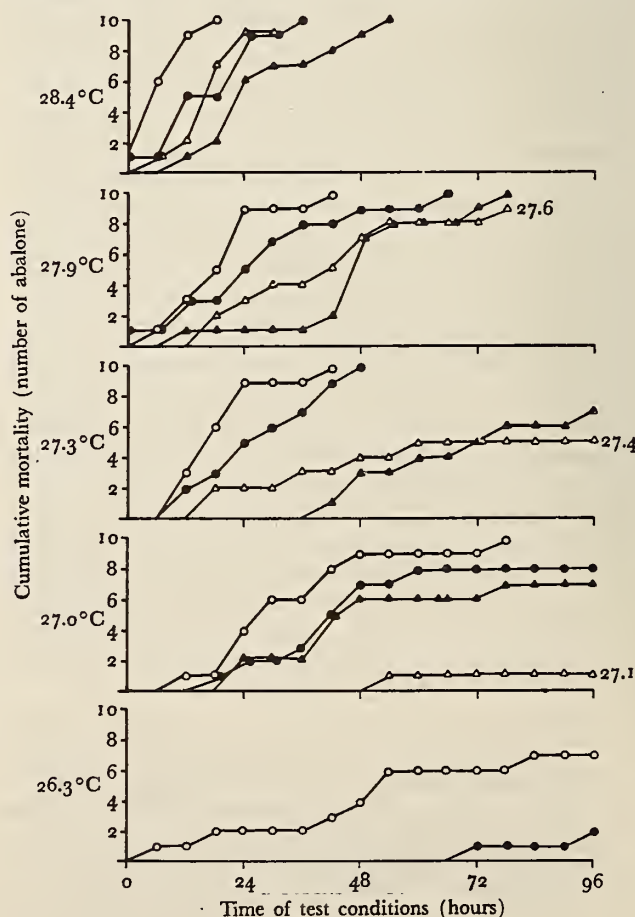
In the main experiments, non-tidal test abalone were held for 96 hours at test seawater temperatures ranging from 24.7 to 29.4°C. Seawater temperatures were raised from the acclimation temperatures to the test temperatures within one hour. Control groups of animals were observed at 11.5 and 16°C, respectively. Air temperatures encountered by abalone during tidal exposure to air averaged 14.8°C over 11.5°C water, 17.5°C over 16°C water, and 21.2 to 21.9°C over the test water of 26.5 to 28.5°C. Within any one test group, the air temperature varied up to $\pm 1.2^\circ\text{C}$, but water temperatures varied only $\pm 0.2^\circ\text{C}$.

Throughout the 96 hour tests, each abalone was observed and gently prodded at 6-hour intervals to determine its ability to hold to a surface. Following the methods of EBERT (1974), loss of the ability of an abalone to hold to

a surface constituted "ecological death" during the experiment. Abalone which lost the ability to hold in tests during the second phase of the experiment were returned to 16°C to check for recovery. At each observation period, systematic notes were made of the abalone's behavior, checking for such things as shell orientation, tentacular response, spawning, body turgor, and unusual behavior. Usually 10, and in a few cases 20, abalone were tested in each group. A total of 418 abalone, including control groups, were used in this experiment. Probit analysis of the mortality data was used in accordance with Standard Methods (1976). Other statistical treatments are explained in the Results.

RESULTS

The time courses of mortality for all the test temperatures during the second phase and for some of the test temperatures during the first phase of the experiment are shown in Figure 1. The response to elevated temperatures is ex-



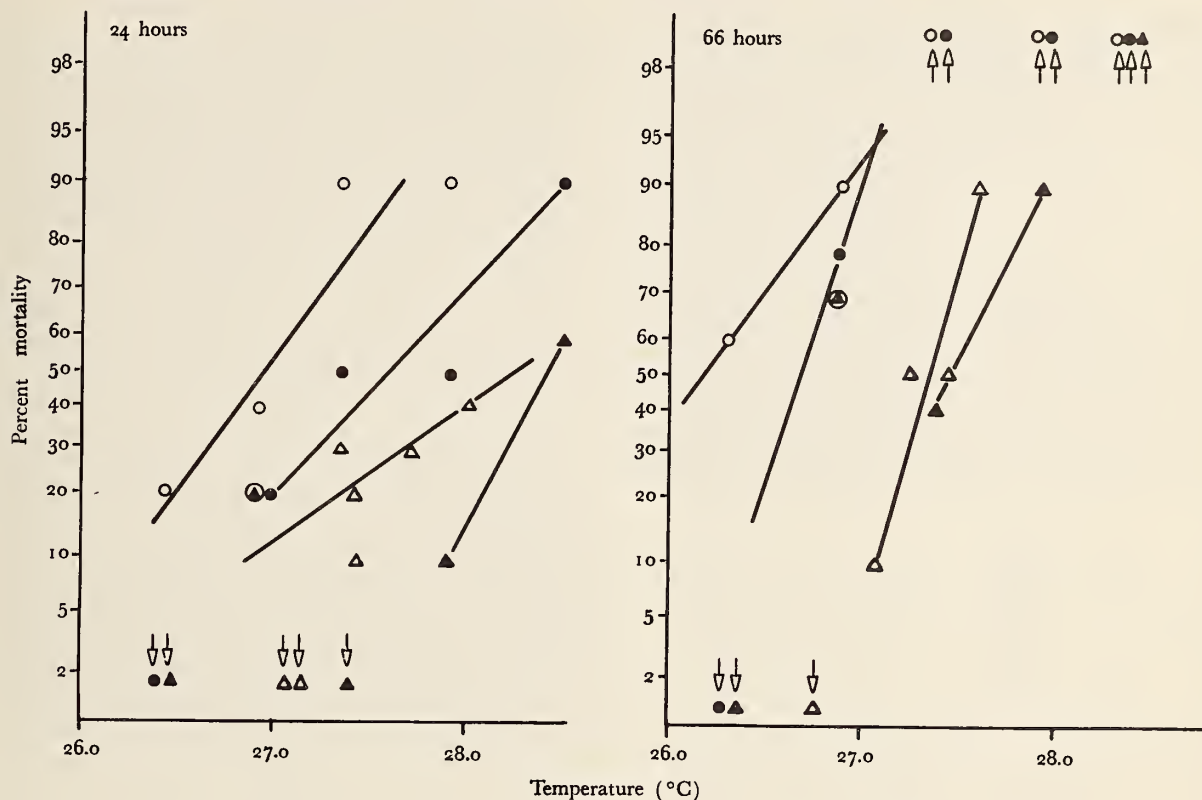


Figure 2

Representative probability plots of the mortality of black abalone from the four experimental groups at 24 and 66 hours into the 96 hour test period. Note that mortality is plotted on a probability scale. Test conditions which resulted in either no or 100% mortality are indicated by arrows. Probit regression lines were fitted by eye and

ignore the spurious result from the 16°C acclimated tidal group tested at 27.0°C (circled). ○ = 11°C acclimated non-tidal group; ● = 11°C acclimated tidal group; △ = 16°C acclimated non-tidal group; ▲ = 16°C acclimated tidal group

tremely abrupt in that, when lethal temperatures are reached, the temperature range from no mortality to 100% mortality is only about 1.0°C for any one experi-

mental group. As might be expected, the precise temperature at which a given response occurred depended upon the experimental group, but based on the results of this experiment, it is predicted that no mortality would occur at or below 25°C and 100% mortality would occur at or above 28.0°C for all experimental groups.

The mortality data for each six hour observation period were plotted on a probability scale versus temperature (see Figure 2 for examples from the 24 and 66 hour observations). These plots show good co-linearity of points necessary for probit analysis. The only exception to co-linearity occurred in the 16°C acclimated tidal group at 27°C, which experienced high mortality relative to animals of the same group at other temperatures. Careful observation of the animals at 27°C did not reveal any

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Figure 1

The time course of mortality of *Haliotis cracherodii* from the four experimental groups at five test temperatures. Only some of the data for the 16°C acclimated, non-tidal group, which was run as a separate experiment, are included for the most comparable temperatures. ○ = 11°C acclimated non-tidal group; ● = 11°C acclimated tidal group; △ = 16°C acclimated non-tidal group; ▲ = 16°C acclimated tidal group

abnormalities. All of the animals at 27° C were males (by chance), however, the mortality rates of males was not significantly different from that of females ($\chi^2 = 1.1493$ for the first phase, and $\chi^2 = 0.4736$ for the second phase, 1 d.f., $p > 0.10$). Thus, the anomalous response of the 27° C animals remains unexplained, and these data were omitted from the probit analysis below. Probit regression lines were fitted by eye to determine the temperature which would result in 50% mortality for each of the 4 experimental conditions at each 6-hour period. For example, at 24 hours the non-tidal group acclimated to 11° C had a 50% mortality rate at 27° C (see Figure 2).

The temperatures at 50% mortality derived from the probability plots were used to generate the curves of time to 50% mortality versus temperature shown in Figure 3. As determined by the temperature producing an effect in

50% of the sample (ET_{50}), the 11° C acclimated non-tidal group had the lowest thermal tolerance (96 hour $ET_{50} = 26.1^\circ \text{C}$), followed by the 11° C tidal group (96 hour $ET_{50} = 26.6^\circ \text{C}$), and by the 16° C tidal group (96 hour $ET_{50} = 27.2^\circ \text{C}$). The 16° C acclimated non-tidal group was tested in a separate experiment under slightly different conditions earlier in the summer. This group had the highest 96 hour ET_{50} (27.4°C); however, it had a thermal tolerance intermediate between the 11° C acclimated tidal and the 16° C acclimated tidal groups for the test period from 6 to 65 hours. Mortality between the 11° C acclimated tidal, the 11° C acclimated non-tidal, and the 16° C acclimated tidal groups was significantly different ($\chi^2 = 7.789$, 2 d.f.; $p < 0.05$) (Table 1). Abalone acclimated to 16° C had fewer deaths than those acclimated to 11° C ($\chi^2 = 5.56$, 1 d.f.; $p < 0.05$) (Table 2), and abalone exposed to a tidal cycle had fewer deaths than those constantly submerged ($\chi^2 = 6.091$, 1 d.f.; $p < 0.05$) (Table 3).

The revival of abalone in the first phase of the experiment (the 16° C acclimated non-tidal) group was not tested, but revival of abalone failing to hold a surface in the second phase of the experiment was only 15.8%. Most of the abalone that revived were animals which lost the ability to hold during the first 24 hours at temperatures

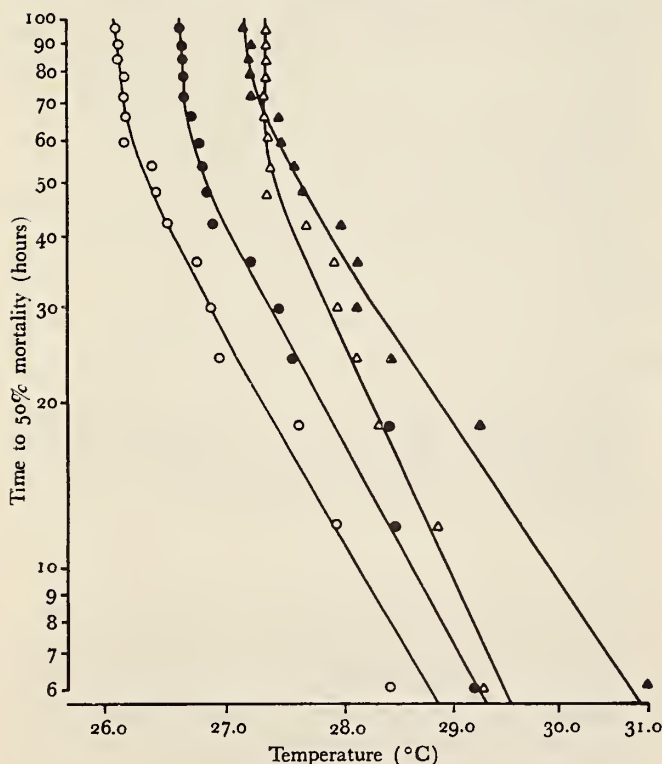


Figure 3

Temperature tolerance of *Haliotis cracherodii* from the four experimental groups. The ET_{50} temperatures at each 6 hour observation were determined from the probability plots, as shown in Figure 2. The 16° C acclimated non-tidal group was tested first and the other 3 groups were tested together at a later date. Note that time to 50% mortality is plotted on a log scale. ○ = 11° C acclimated non-tidal group; ● = 11° C acclimated tidal group; △ = 16° C acclimated non-tidal group; ▲ = 16° C acclimated tidal group

Table 1

Contingency table comparing the mortality of the 16° C acclimated tidal, 11° C acclimated tidal, and 11° C acclimated non-tidal groups. The groups were significantly different ($p < 0.05$; $X^2 = 7.789$, 2 d.f.)

	16T	11T	11NT	Total
No. dead	34 (56.7%)	40 (67.8%)	46 (80.7%)	120
No. alive	26 (43.3%)	19 (32.2%)	11 (19.3%)	56
Total	60	59	57	176

Table 2

Contingency table comparing the effect of acclimation to 16° C or 11° C on mortality. The two conditions had significantly different mortalities ($p < 0.05$; $X^2 = 5.56$, 1 d.f.)

	16° C	11° C	Total
No. dead	34 (56.7%)	86 (74.1%)	120
No. alive	26 (43.3%)	30 (25.9%)	56
Total	60	116	176

Table 3

Contingency table comparing the effect of continuous submersion and a simulated tidal cycle on mortality. The two conditions had significantly different mortalities ($p < 0.05$; $X^2 = 6.091$, 1 d.f.)

	Tide	No tide	Total
No. dead	74 (62.2%)	46 (80.7%)	120
No. alive	45 (37.8%)	11 (19.3%)	56
Total	119	57	176

above 27.5° C. Abalone that failed to hold later in the test period did not recover.

Sperm spawned during the experiment showed no motility above 27.0° C, whereas sperm motility appeared unaffected below 27.0° C. Because only one female spawned during the experiment, it was not possible to determine the effects of heat stress on eggs.

The behavioral sequence during heat stress in black abalone was fairly consistent, although all abalone did not exhibit all stages of the response. The first sign of stress was a general loss of body turgor. Secondly, the shell was uplifted from the substrate, usually beginning with a 1-2 cm lift of the anterior end, eventually resulting in elevation of the entire shell 4 cm off the substrate ("gaping"). Gaping was often followed by a spreading of the epipodium 1-2 cm beyond the edge of the shell. Loss of the ability to hold a surface usually followed epipodial spreading, although failure to hold did occur at various points. Loss of tentacular responsiveness followed failure to hold. Spawning by males occurred more frequently at test temperatures than at the control temperatures and did occur at every observation period during the stress period. Abalone under heat stress did not move to escape the warm water.

DISCUSSION

EBERT (1974) used methods similar to those in the present study to determine thermal tolerance of *Haliotis rufescens*, and his data were used to estimate the 96 hour ET_{50} of *H. rufescens* for comparison with our results for *H. cracherodii*. The thermal tolerance of the intertidal species, *H. cracherodii*, was higher than that of the subtidal species, *H. rufescens*. The 96 hour ET_{50} temperature for continuously submerged abalone was 26.1° C for *H. cracherodii*

acclimated to 11.5° C, whereas for *H. rufescens* acclimated to 10° C it was about 24° C. Acclimation to warmer temperatures resulted in a small but significant increase in thermal tolerance in both species. For *H. cracherodii* raising the acclimation temperature from 11.5° C to 16.0° C increased the ET_{50} about 1.3° C, whereas for *H. rufescens* raising the acclimation temperature from 10° to 20° C increased the ET_{50} about 1° C.

Black abalone at the tidal level of 0.3 to 1.0 m above mean lower low water are not exposed to elevated temperatures for 96 hours before being inundated with cool seawater: a 6-12 hour exposure would be typical. For these shorter exposures, the ET_{50} values are about 1-1.5° C higher than for the 96 hour exposures and fall in the 28-31° C range (Figure 3). As expected, *Haliotis cracherodii* exposed to a fluctuating thermal regime, which greatly reduced the "heat dose" during the test period, had a much higher survivorship than those exposed to continuous stress (37.8% versus 10.3% alive; Table 3). Surprisingly, however, the increase in ET_{50} value for the 11° C acclimated group in the fluctuating regime was small (only 0.5° C), and the ET_{50} values for all experimental groups were within a narrow 2° C range of each other.

The response to heat stress in *Haliotis cracherodii* in all test groups was extremely abrupt with the difference between 0 and 100% mortality in 96 hours being only about 1° C once lethal temperatures were reached. Therefore, small changes in temperatures near the critical zone of temperatures above 25° C may have serious consequences for black abalone. WOLCOTT (1973) found similarly abrupt survivorship responses to elevated temperatures in several species of intertidal acmaeid limpets, with the difference between 0 and 100% mortality in short tests also being only 1-2° C. The behavioral response of black abalone to thermal stress, especially the uplifted shell gaping and epipodial spreading, would be adaptive for evaporative cooling, but this would increase desiccation and leave the abalone more vulnerable to predation. Because intertidal organisms are regularly exposed to temperature extremes, and because limpet-like snails have a foot which presents a large surface area in contact with the substrate and little capacity for insulation, the ability to tolerate temperatures very near the ET_{50} point has high adaptive value.

SUMMARY

1. The thermal tolerance of adult *Haliotis cracherodii* acclimated to 11.5° C and 16° C was determined for animals exposed to constant temperatures during con-

tinuous submergence and to fluctuating temperatures during a simulated tidal cycle. Loss of the ability of an abalone to hold a surface constituted "ecological death."

2. The response to elevated temperature was extremely abrupt in that, when lethal temperatures are reached, the temperature range from no mortality to 100% mortality was only about 1.0° C for any one experimental group.
3. As determined by probit analysis, the 96 hour ET_{50} value of the 11° C acclimated/non-tidal group was 26.1° C, that of the 11° C acclimated/tidal group was 26.6° C, and that of the 16° C acclimated/tidal group was 27.2° C. The 16° C acclimated/non tidal group in separate tests had a 96 hour ET_{50} value of 27.4° C, but it had a thermal tolerance intermediate between the 11° C/tidal and the 16° C/tidal groups for the test period from 9 to 65 hours.
4. The revival of abalone returned to 16° C after failing to hold during part of the experiment was only 15.8%.
5. Sperm spawned during the tests were motile below 27° C, but non-motile above 27° C.
6. The behavioral response of *Haliotis cracherodii* to heat stress was described.

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